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SOME PHARMACOKINETIC AND METABOLIC FACTORS AFFECTING THE NEONATAL TOXICITY OF CHLORINATED HYDROCARBONS FOUND IN THE GREAT LAKES

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16. ABSTRACT

Industrialization has resulted in the emission of a wide variety of organic chemicals into the biosphere. Despite the original source of the organic contaminant, these chemicals have managed to enter drinking water supplies and bioaccumulate in food chains. Of particular interest are the organic contaminants which have been identified in fish and wildlife in the Great Lakes Basin. As this waterway system functions as an important source of drinking water for North Americans, concern has been raised with respect to toxicity and bioaccumulation of these chemicals. Although a vast number of environmental pollutants have thus far been identified, this chapter will focus on just ten chemicals, principally chlorinated benzenes, octachlorostyrene and hexachlorobutadiene. The adult, fetal and neonatal toxicities of these compounds are discussed with emphasis on metabolic and pharmacokinetic factors which influence them.

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Abstract:

Industrialization has resulted in the emission of a wide variety of organic chemicals into the biosphere. Regardless of the original source, these chemicals have managed to enter drinking water and to accumulate in food chains. Of particular interest are the organic contaminants which have been identified in fish and wildlife in the Great Lakes Basin. As this waterway system is an important source of drinking water for North Americans, the toxicity and bioaccumulation of its contaminants have been investigated. Although a vast number of environmental pollutants has thus far been identified, this chapter will focus on just ten nonpolar chemicals, principally chlorinated benzenes, octachlorostyrene and hexachlorobutadiene. The adult, fetal and neonatal toxicities of these compounds are discussed with emphasis on metabolic and pharmacokinetic factors which influence them. A summary of the organic contaminants to be discussed is listed in Table 1.

Introduction

General Chemistry and Ecology:

A useful classification of chlorinated aromatic hydrocarbons based on chemical polarity and metabolism has been developed by Matthews and Kato (1979) (Table 2). Classification is determined by whether a compound possesses a polar functional group which facilitates rapid renal excretion; and whether oxidative metabolism is required before the mammalian system can clear it via the bile or urine. With high polarity Type I compounds rapid renal excretion of the parent compound or its conjugates ensures a short biological half-life and prevents bioaccumulation. High tissue levels of Type I compounds are often observed in the blood and kidney (the prime excretory organ for Type I compounds).

Type II molecules have functional groups of intermediate polarity such as the oxygen of the dibenzo-p-dioxin bridge (Table 2). These lipid soluble molecules often are found in high levels in liver and fat as they require hepatic oxidation before biliary excretion of an oxidized and conjugated derivative.

Type III chlorinated aromatics have no polar functional groups and are very resistant to cytochrome P-450 mediated oxidation. They display a long biological half-life, accumulate to very high levels in fat and bioaccumulate in food chains. One measure of a compound's solubility in lipids is its n-octanol to water partition coefficient. A high value implies that a compound will be deposited in the body's fatty tissue and if not rapidly metabolized it may bioaccumulate. The log p values for octachlorostyrene, hexachlorobenzene, pentachlorobenzene, 1,2,4,5-tetrachlorobenzene, 1,2,4-trichlorobenzene and hexachloro-1,3-butadiene are 6.8 to 7.7, 6.18 to 6.43, 5.63, 4.93, 4.23 and 4.79 respectively. The corresponding log p values for the less substituted dichlorobenzene, monochlorobenzene and benzene are 3.38, 2.84 and 2.13 respectively (Birnbaum, 1985).

The concentration of various chlorinated benzenes in water, sediments, and trout in two Great Lakes is presented in Table 3 (Oliver and Nicol, 1982). In addition, values for human drinking water and the mouth of the Niagara River (a heavily contaminated aquatic system) are included (Durham and Oliver, 1983). In Great Lake water and sediment, 1,2,4-trichlorobenzene is often found in relatively higher concentrations than the other chlorinated benzenes. The tendency for hexachlorobenzene (HCB) to bioaccumulate in food chains is shown clearly by the high levels found in trout from both Great Lakes, particularly Lake Huron. In this aquatic ecosystem 1,2,4,5-tetrachlorobenzene

does not show as strong a tendency towards bioaccumulation as one might expect based on its chemical properties and how slowly this compound is excreted by both rabbits and rats (Tables 4 and 5).

Pharmacokinetic Features:

Among the chlorinated benzenes the number and position of the halogen substituents greatly influence the compound's metabolism, excretion and toxicity. Rabbits metabolize and excrete chlorobenzenes in their urine at rates which vary at least fifty-fold (Table 4). Lesser chlorinated species, particularly those which possess two adjacent hydrogens are oxidized and excreted at faster rates (Williams, 1959). Molecules with only single isolated hydrogen substituents are metabolized and excreted slowly. These general rules work well in predicting relative rates of oxidation and excretion. However, for 1,2,4,5-tetrachlorobenzene and pentachlorobenzene (QCB) the opposite of the general rule is observed. QCB is metabolized faster than 1,2,4,5-tetrachlorobenzene even though the latter has two, albeit separate, positions for oxidative attack. attack.

In feeding studies with chlorinated benzenes, large amounts of the test compounds accumulate in fat of rats given either 1,2,4,5-tetrachlorobenzene, QCB or HCB (Table 5). Rats can dispose of dietary 1,2,3,4- and 1,2,3,5-tetrachlorobenzene fairly readily without building up substantial fat levels or body burdens of these chemicals (Chu et al., 1983). Both of the compounds have two ring positions open for oxidative attack even though they are separated by one chlorine in the case of 1,2,3,5 tetrachlorobenzene.

Metabolic and Biochemical Features:

Large differences in the biochemical effects of two chlorinated benzenes are shown in Table 6. Equimolar doses of the two chemicals were given at the

LD50 value of the more toxic isomer, 1,2,4-trichlorobenzene. At this dose level, no DNA damage was caused by either compound. 1,2,4-Trichlorobenzene caused a 50% decrease in hepatic glutathione and increased serum alanine aminotransferase (SGPT) activity by threefold. Increases of 20% in cytochrome P-450 content and 130-fold in ornithine decarboxylase activity resulted from the treatment with 1,2,4-trichlorobenzene.

1,2,4-Trichlorobenzene possesses two vicinal hydrogens, can form reactive intermediates, such as epoxides, and induce the toxic biochemical sequelae of these short-lived reactive species. Thus hepatic glutathione is decreased by conjugation with reactive intermediates. SGPT is elevated because either cell membrane damage or hepatic cell death has occurred following 1,2,4-trichlorobenzene treatment. Cell death and the resulting mitotic stimulation are the likely cause of the enormous increase in hepatic ornithine decarboxylase observed.

As 1,2,4,5-tetrachlorobenzene lacks vicinal hydrogens, reactive intermediates will not be formed at a high rate. Thus no glutathione depletion or increase in serum alanine aminotransferase activity were expected or observed. 1,2,4,5-Tetrachlorobenzene poses a far greater risk for chronic toxicity (e.g., see excretion kinetics and bioaccumulation of this isomer in Tables 4 and 5). In contrast 1,2,4-trichlorobenzene poses a greater risk for acute toxicity because of its propensity to form reactive intermediates.

In the upcoming sections on compound specific toxicity, compounds will be presented in order of decreasing degree of chlorination and decreasing n-octanol to water partition coefficient.

Adult and Neonatal Toxicology of Ten Non-Polar Chlorinated Hydrocarbons

1. Octachlorostyrene:

Industrialization has resulted in the emission of octachlorostyrene

(OCS) into the biosphere, although the precise source of contamination remains to be established. In the production of magnesium, Lunde and Bjorseth (1977) reported that OCS was generated and subsequently released into the biosphere. OCS is one of the organic contaminants emitted in the combustion of polyvinyl chloride, a compound used within the construction industry in pipelines for drinking water, electrical installations, ventilation drums and roof gutters (Ahling et al., 1978). Ofstad et al. (1978) found that in certain industrial high temperature reactions involving carbon and chlorine, OCS was a byproduct and a potential contaminant. As a result of these industrial processes OCS has been identified in tissues of fish (Lunde and Baumann-Ofstad, 1976; Kuehl et al., 1976; Ofstad et al., 1978), in carcasses and eggs of birds (Reichel et al., 1977) and in human blood (Lunde and Bjorseth, 1977). OCS was also identified as one of the organic contaminants present in the Great Lakes (Kuehl et al., 1976; Konasewich et al., 1978). OCS is chemically similar to HCB in structure. It can be regarded as a fully chlorinated vinylbenzene. OCS is highly fat soluble with a log p of 6.8-7.7 (Chu et al., 1986). Only 7% of an iv dose of OCS was excreted in the feces and none in the urine during the next 7 days. Based on this, a biological half-life of about 70 days would be expected, typical of a Type III halogenated aromatic.

Although OCS is a known environmental pollutant, few data are available on its potential toxicity to mammals. In 1975, Strik and Koeman demonstrated that OCS produced porphyria in Japanese quail and adult rats. The observed porphyria in adult rats was associated with hepatic megalocytosis, basophilic spots and hepatocyte hypertrophy, which are indices of abnormal liver function (Strik and Koeman, 1975). In a subsequent study, OCS was found to induce an

accumulation of porphyrin in humans in the Netherlands (Strik, 1978). Similarly, several investigators have reported that OCS produced liver hypertrophy as well as histological alterations in adult rat hepatic and thyroid tissue (Chu et al., 1982a; Holme and Dybing, 1982a). OCS stimulated a general increase in liver weight as well as a specific induction of hepatic cytochrome P-450 and the monooxygenase drug metabolizing enzymes (Chu et al., 1982a; Holme and Dybing, 1982b). In addition, Holme and Dybing (1983) found that OCS stimulated the activities of drug metabolizing enzymes, such as epoxide hydrolase and glutathione S-transferase, which are not cytochrome P-450 based but which might exert a profound effect on the toxicity of environmental compounds.

In an extensive study, Kacew et al. (1986) administered doses of 50, 100, 200 or 400 mg/kg/day of OCS by gavage to pregnant Sprague-Dawley rats from day 6 through day 15 of gestation. On day 21 of gestation all surviving dams were sacrificed and pups removed by caesarean section for teratological evaluation. In contrast to adult non-pregnant rats (Holme and Dybing, 1982a), treatment of pregnant rats with OCS resulted in increased mortality (Table 7), especially in the 200 and 400 mg/kg groups approximately 8.8 days after initiation of treatment while in the greater (400 mg/kg) dose group death occurred after 7.5 days. At necropsy these animals displayed fatty metamorphosis of the liver, hemorrhagic enteritis, toxic changes in the placenta and fetuses as well as myocardial ischemia. Maternal liver weight whether expressed either as an absolute (g) or as a percent of total body weight increased significantly in rats treated with either 200 or 400 mg/kg OCS. While the elevation in absolute liver weight was quantitatively higher in rats given the 400 mg/kg dosage, the observed rise in hepatic weight was also significant from all the other treatment groups when expressed as a

percent of body weight. The weights of maternal spleen, heart, kidney and brain were not markedly different in any of the OCS-treated dams when compared to corresponding control values.

An important characteristic associated with exposure to OCS is the ability of this contaminant to induce hepatic drug metabolizing enzymes in the non-pregnant animal (Holme and Dybing, 1982a; Chu et al., 1982a). Pregnant rats have a lower constitutive hepatic cytochrome P-450 content and are less inducible than are nonpregnant females (Dean and Stock, 1975; Guenthner and Mannering, 1977; Kitchin and Ebron, 1983b). Metabolism of aminopyrine per mg microsomal protein (46%), per g liver (58%), per cell (79%), per body weight (62%) or per animal (82%) is less for pregnant rats than for nonpregnant females regardless of how the data are expressed (Dean and Stock, 1975). Irrespective of the OCS treatment regimen, there were no marked alterations in hepatic protein or in the activities of microsomal aniline hydroxylase and aminopyrine N-demethylase in pregnant dams. Kacew et al. (1986) demonstrated mortality caused by OCS was enhanced in the absence of liver microsomal enzyme induction as only pregnant dams seemed to die. Data thus suggest that induction of hepatic drug metabolizing enzymes confers a potential protective mechanism against OCS-inflicted lethality.

Examination of the pups for developmental abnormalities revealed certain anomalies (Table 7). While hydronephrotic kidney was observed in all treatment groups, undersized pups were noted only in the 400 mg/kg OCS group. Histologically, small changes also occurred in the kidneys of pups. These changes consisted of an increased eosinophilia of the proximal epithelium, dilated and flattened epithelium and loss of cell nuclei. The lesions were confined to the 200 and 400 mg/kg groups with two and one lesions,

respectively. Bone marrow had a reduction in overall density with a shift to increased levels of myelopoiesis with reduced erythropoiesis in the 200 and 400 mg/kg treatment groups (Kacew et al., 1986).

A dose-related accumulation of OCS residues was found in fetal liver, brain and whole fetus. The hepatic residue levels were approximately 1.5 to 2-fold higher than those present in the whole fetus. In general, the OCS concentration found in adult tissue was also dose-related in heart, kidney, perirenal fat and brain. In liver, the highest residue level was noted in animals administered 200 mg/kg compound and this residual amount did not increase further when OCS dosage was elevated. In spleen, the residue level increased proportionately from adult rats treated with 50 mg/kg to 100 mg/kg, reached a plateau between 100 and 200 mg/kg and was followed by an approximate 2-fold rise when 400 mg/kg was administered. Chu et al. (1982b) also reported that OCS accumulated to the greatest extent in perirenal fat of adult male and non-pregnant female rats. The dose-related increase in OCS residue accumulation was associated with more severe teratologic and histopathologic alterations. The findings by Chu et al. (1982a) that in rats OCS remains in the unmetabolized form suggests that the parent compound and not a metabolite may be responsible for the observed toxicity in the dam and fetus.

At present the effects of OCS on newborn animals remain to be determined. However, chronic studies were conducted by Chu et al. (1984, 1986) starting with weanling (21 day-old) rats. Supplementation of food pellets with OCS concentrations ranging from 0.05 ppm to 50 ppm for either 13 weeks or 1 year resulted in increase in liver weight in male and female animals. As expected, hepatic tissue hypertrophy was accompanied by elevation in the activities of microsomal aniline hydroxylase and aminopyrine N-demethylase. As in the case of pregnant rats, morphological alterations were noted in liver, kidney and bone

marrow. Chu et al. (1984b) found that after 13 weeks OCS altered bone marrow as evidenced by myeloid hypertrophy and erythroid cell depression, while in kidney there were glomerular adhesions associated with proteinaceous casts in the lower nephron and focal tubular degeneration. Continuation of OCS feeding for 1 year resulted in hepatic cytoplasmic enlargement, aniksokaryosis and aggregated basophilia. In the thyroid, changes consisted of reduced colloid density, focal collapse of follicles and increased epithelial height (Chu et al., 1986). In addition to dose-related morphological alterations, Chu et al. 1984b, 1986) observed a dose-dependent accumulation of OCS in fat and liver of weanling rats. In pregnant rats OCS increased mortality but did not induce adult hepatic microsomal drug-metabolizing enzymes. In neonates OCS accumulates in mammalian tissues and is potentially toxic as manifested by morphologic alterations.

2. Hexachlorobenzene (HCB):

HCB arises as a production contaminant of chlorinated solvents, pentachlorophenol, dachthal and pentachloronitrobenzene (Verschueren, 1983). HCB has been used as a fungicide, a wood preservative, in the seed treatment industry, in aluminum manufacturing and in the manufacture of pyrotechnics and synthetic rubber (Vershcueren, 1983). In the mid-1970's the annual U.S. production of HCB (primarily as an inadvertent synthesis contaminant) was approximately four million pounds per year (Courtney, 1979). Through the production of HCB as a by-product, its widespread use in the chemical industry, and inadequate waste disposal procedures, residues have been found in fish (Zitko, 1971; Johnson et al., 1974), wildlife (Vos et al., 1968) and poultry and dairy products (Luquet et al., 1972). A biomagnification factor of 172

(fish HCB level/water HCB level) was found in one ecosystem in Mississippi (Courtney, 1979).

In an extensive study, Vos et al. (1971) fed varying amounts of HCB to Japanese quail for 90 days. In birds receiving from 5 to 80 ppm HCB, this chemical increased liver weight, and was found to produce porphyria and hepatic damage. However, in the 80 ppm group there was also reduced production and volume of eggs, kidney damage, tremors as well as mortality. Similarly, Schwetz et al. (1974) found that 20 ppm administered to adult Japanese quail decreased survival of chicks and increased liver weight of adult birds. Recently, Carpenter et al. (1985) reported that the HCB-induced elevation in hepatic porphyrins of Japanese quail was associated with a rise in the activity of liver deltaaminolevulinic acid synthetase. Although a HCB metabolite, tetrachlorohydroquinone (TCHQ), did not produce a marked porphyria; the combination of HCB and TCHQ resulted in a synergistic response and a more severe porphyria. These findings suggested that metabolites do not play a primary role in the generation of porphyria but possess the ability to exacerbate the effects of HCB on the heme biosynthetic pathway in quail (Carpenter et al., 1985).

In rats the symptoms of acute HCB poisoning include tremors, weakness, ataxia and paralysis (Ockner and Schmid, 1961). Administration of HCB was reported to increase rodent kidney and liver weight as well as nervous system disturbances (Ockner and Schmid, 1961; Leoni and D'Arca, 1976). It is worthwhile to note that a dietary intake of 100 mg/kg/day HCB enhanced rodent mortality (Ockner and Schmid, 1961). Female rats are more susceptible to HCB-induced porhyria and death (Kuiper-Goodman et al., 1977, Kimbrough and Linder, 1974). In addition to an increase in liver weight, HCB was found to produce hepatic damage characterized by enlargement of hepatocytes which contained discrete eosinophilic laminated cytoplasmic bodies (Medline et al.,

1973). Hepatic cellular enlargement in the centrilobular region, increase in smooth endoplasmic reticulum, and extensive vacuolation were caused by HCB (Kuiper-Goodman et al., 1977). HCB administration resulted in induction of liver cytochrome P-450, aniline hydroxylase and aminopyrine N-demethylase (Wada et al., 1968; Grant et al., 1974). As in the case of Japanese quail, HCB was found to disrupt the hepatic heme biosynthetic pathway in rats as evidenced by an accumulation of hepatic porphyrin and increased excretion of urinary porphyrin (Grant et al., 1974; San Martin de Viale et al., 1970, De Matteis et al., 1961).

Although it appears that liver is the primary target tissue for HCB toxicity, this chemical also exerts toxic actions elsewhere. HCB administration to rats, guinea pig, mice and rabbits was found to produce neurological disturbances. In particular, HCB produced hyperexcitability, tremor and paresis (Ockner and Schmid, 1961; De Matteis et al., 1961). However, pathological evidence of central nervous system damage was not apparent (De Matteis et al., 1961). Their nervous system disturbances occurred just prior to death (Ockner and Schmid, 1961; De Matteis et al., 1961). These findings in four species are in marked contrast to the symptoms noted in humans where central nervous system changes do not appear to occur (De Matteis et al., 1961).

Elder et al. (1976) demonstrated that HCB increased the activity of liver delta-aminolevulinic acid synthetase, decreased the activity of uroporphyrinogen decarboxylase and increased formation and excretion of porphyrins. Furthermore in the presence of pentachlorophenol, a HCB metabolite, the onset of HCB-induced porphyria was accelerated. Debets et al. (1980) also suggested that the porphyrinogenic action of HCB in rats was augmented by metabolites. However, in

vivo administration of pentachlorophenol and chlorophenols per se to rats exerted no porphyrinogenic actions (Wainstok de Calmanovici and San Martin de Viale, 1980). In contrast, incubation of hepatic homogenates with pentachlorophenol, TCHQ or tetrachlorobenzenes resulted in increased porphyrin levels and decreased uroporphyrinogen decarboxylase (Billi et al., 1986). These findings demonstrate that HCB metabolites may play a vital role in the HCB-inflicted damage to the heme biosynthetic pathway in mammals. Since HCB metabolites exacerbate the manifestations of parent HCB poisoning in animals, the possible role of these metabolites in human adverse effects requires further investigation.

In humans, the chronic consumption of HCB leads to a disturbance in the heme biosynthetic pathway (Cam and Nigogosyan, 1963). The main human symptoms are skin lesions, porphyrinuria, photosensitization, weight loss, hepatomegaly and hypertrichosis (Cam and Nigogosyan, 1963; Schmid, 1960). Some human milk, blood and fat levels of HCB in occupationally- and environmentally-exposed individuals are presented in Table 8. The range of values reported is enormous, from two orders of magnitude for fat, to three orders of magnitude for both milk and blood. The fat, blood and milk levels of HCB in the Turkish poisoning incident of the late 1950's are not known. The daily intake of HCB has been estimated to have been 50-200 mg/kg HCB per day in poisoned Turkish individuals (Greim and Wolff, 1984). In a follow-up study conducted 25 years after HCB poisoning, the mean human milk HCB level was 510 ppb. In a recent epidemiologic study, humans with 170 ppb HCB in blood were described as clinically healthy. In the North American Great Lakes region, values ranged from 0.5 to 100 ppb HCB in human milk.

HCB poisoning involving 3,000-5,000 people with approximately a 10% mortality rate occurred in Turkey in the late 1950's. This poisoning

epidemic affected children most severely. The disease state was often called "pink sore" or pembe yara. In some Turkish villages consuming fungicide treated grain, a 95% mortality rate in affected children was observed. Virtually all children between the ages of 2 months and 5 years were killed in some villages. HCB toxicity was observed in humans who ingested HCB treated seed grain before it became known that the general environment was contaminated with this chemical. Historically animal experiments were conducted with HCB after humans had been poisoned.

In Turkey, rheumatoid arthritic changes were found in many HCB-affected children. The incidence increased from an initial 36% to 55% in a subsequent study five years later (Dogramaci, 1964; Dogramaci et al., 1962). Some osteoporosis, resorption of digits, bone thinning, and failure of hands to grow to normal adult size were observed (Cripps et al., 1980).

Porphyria cutanea tarda (PCT) was a classic symptom observed in many of the HCB-poisoned Turkish population (Peters et al., 1982). PCT was seen frequently in children aged 6 to 16. Only 10% of PCT cases were older than 16. One-third of the patients had hepatomegaly, a common sign of exposure to high levels of chlorinated hydrocarbons. Prior to death a peculiar syndrome of a cutaneous human porphyria was observed particularly in children under the age of 4 (Pestel, 1963). With respect to three of the biological effects of HCB poisoning, neonatal death, hepatomegaly and porphyria cutanea tarda, both man and experimental animals respond similarly.

Administration of HCB at doses of 80 to 120 mg/kg/day to pregnant Sprague-Dawley rats from day 6 to 16 of pregnancy produced no fetopathic alterations in day 22 fetuses (Villeneuve and Hierlihy, 1975). Although HCB did not appear to induce teratological alterations, HCB was found to

accumulate in the fetus in a dose-dependent manner. In a recent study, Courtney et al. (1985) demonstrated that administration of HCB to pregnant guinea pigs and hamsters also resulted in the accumulation of HCB in fetal tissues. It is of interest that the concentration of HCB in the hamster fetus was five-fold greater than in the guinea pig fetus. These studies clearly show that HCB readily crosses the placental barrier but that no apparent adverse effects are evident on fetal development.

Transfer of chlorinated hydrocarbons from the dam to the neonate may take place via the placenta or via lactational exposure following birth. With HCB, transfer in the milk can be the major route of exposure in both man and the rat. In rodents the lactational exposure to HCB is so great that toxic sequalae are observed in nursing experimental animals. HCB-treated dams which do not show any signs of clinical toxicity will give birth to normal pups which will die during lactation. The newborn pups are overwhelmed by HCB residues transferred in their mother's milk. In a massive depletion of the maternal body burden, the maternal mouse fat level of HCB is decreased by 95% during the 20-day lactation period (Courtney and Andrews, 1985). This is an important example of chlorinated hydrocarbon excretion in the milk and accumulation in the offspring. As the role of HCB metabolites in the observed HCB-induced liver damage has been documented for adults (Debets et al., 1980), it is evident that metabolic studies should be undertaken in the newborn.

The maternal dietary concentration of HCB which produces death in a female rat or her suckling pups is quite different. Adult females died after dietary exposure to 320 or 640 ppm HCB (Grant et al., 1977). However, suckling newborn rat pups died when their mothers were maintained on diets of only 80 ppm in one study (Grant et al., 1977) and 60 to 100 ppm HCB in a second study (Kitchin et al., 1982). This is a five-fold difference in dietary HCB levels.

Rat pups die from a much lower brain HCB concentration than do adult animals (Kitchin et al., 1982). In addition, Grant et al. (1977) found that weanling rats had enlarged livers and increased hepatic aniline hydroxylase activity. It is evident that the suckling neonatal rat or human is clearly more at risk from death due to HCB than their mothers (Schmid, 1960; Grant et al., 1977).

In an extensive study Mendoza et al. (1975) administered HCB in the maternal diet 2 weeks prior to birth and maintained the dam on this diet for a further 18 days. In suckling 18-day-old rat pups HCB increased liver weight but decreased the weights of kidney, brain, spleen and heart. The elevation in liver weight was associated with an approximate 2.5 fold rise in liver porphyrin levels. In descending order of pup tissue residues, HCB was present in liver > kidney > lung > brain > spleen > heart. This study clearly demonstrated the pattern of HCB accumulation in suckling rats and that liver is a primary target tissue.

In a cross foster experiment, Mendoza et al. (1977) quantitated the relative contributions of placental and lactational exposure to the levels of HCB found in day 21 rat pups (Table 9). When pups were exposed to HCB via the milk only as compared to via the placenta only, a 30-fold higher brain HCB level resulted. This enormous quantitative difference is not due to the post-partum excretion of HCB obtained previously via the placenta. The biological half-life of HCB in rats, 60-100 days (Kuiper-Goodman et al., 1977), is simply too long compared with the 21-day suckling period. However, a cross fostering experiment such as this is somewhat prejudiced in favor of apparent lactational exposure. In the placentally exposed pups, HCB exposure stops 21 days sooner than the lactationally exposed pups. Rat pups increase about 7 fold in body weight from birth to day 21. This body weight increase and subsequent

dilution of HCB could explain partially the low concentration of HCB found in placentally exposed pups.

3. Pentachlorobenzene:

The major use of pentachlorobenzene (QCB) is in the preparation of pentachloronitrobenzene, a fungicide. In addition, QCB is currently being used as a flame retardant. As a consequence of its industrial uses, QCB has been identified in fish in Norway (Ofstad et al., 1978), in effluent from a wastewater treatment plant in California (Sittig, 1985) and in Great Lakes water (Konasewich et al., 1978). At present there is little information available on the toxicity of this contaminant. In a single study QCB did not produce any apparent carcinogenic effects in rats or dogs but was suggested to be carcinogenic in mice (Sittig, 1985). In the rat, treatment with QCB did not induce porphyria (Debets et al., 1981). In addition, incubation with QCB failed to cause an accumulation of porphyrins in a liver cell culture system (Debets et al., 1981).

The clinical signs of acute QCB poisoning were tremors and narcosis (Linder et al., 1980). In their investigation, dietary exposure to 125 and 1,000 ppm QCB were used for males while female rats were exposed to 125, 250, 500 or 1000 ppm QCB. At 500 ppm QCB and higher, hypertrophy of hepatic cells was observed in adult female rats and in the 21 day-old offspring of treated mothers. Males showed this hepatic effect only at 1,000 ppm. Males also showed a small increase in renal hyaline droplet formation at 125 ppm which was much more prominent at 1,000 ppm QCB. In adult rats a decrease in hemoglobin and hematocrit and a increase in white cells were observed at the highest dose tested (1000 ppm) (Linder et al., 1980). In adult females, increases in liver to body weight ratios occurred at 250 ppm QCB and higher, while in males kidney weights were increased at 125 and 1000 ppm QCB. In spite of dietary exposures

to 1000 ppm QCB for 180 days, female rats did not show any increase in liver porphyrins. This shows QCB is remarkably less potent than HCB in inducing porphyria.

Chu et al. (1983) gave 5, 50 or 500 ppm QCB in the diet to rats. The activity of the hepatic enzyme aniline hydroxylase was increased at feeding levels as low as 50 ppm. Pathological changes in the thyroid and lungs were observed at 5 ppm QCB and higher. Pathological changes in the liver were observed at 50 ppm and 500 ppm. This study found QCB induced lesions to be basically similar to those caused by other tetra- and hexa- chlorobenzenes.

When pair-bred with untreated partners, fertility and fecundity of rats was not altered by administration of up to 1000 ppm QCB in the diet (Linder et al., 1980). However, neonatal death occurred in pups nursing from dams fed 500 or 1000 ppm QCB in the diet. Dietary levels of QCB as low as 250 ppm administered to the dams increased the liver to body weight ratios of weanling pups. This dietary exposure level was providing about 18 mg/kg/day of QCB to the lactating mothers.

Khera and Villeneuve (1975) administered QCB to rats during days 6-15 of gestation. Maternal weight loss was found not to be statistically significant although a significant loss of mean fetal weight occurred at 200 mg/kg. Extra ribs were observed in day 21 fetuses from dams given 50, 100 or 200 mg/kg QCB. At 200 mg/kg unossified or nonaligned sternebrae were seen in 31% of treated offspring compared to 4% in the controls. The fetal brain and liver levels of QCB were about 40% of the maternal residue levels (Villeneuve and Khera, 1975).

QCB crossed the placenta and accumulated in fetal liver, brain and whole body in a dose-dependent manner. The administration of QCB to pregnant mice resulted in accumulation of QCB in fetal tissue without causing any

associated developmental changes (Sittig, 1985). In summary it would appear that QCB readily crosses the placental barrier and that relatively small doses are not harmful to the fetus. As the lactational exposure to QCB reaches a critical level, organ weight changes and death can occur in the nursing pups.

Khera and Villeneuve (1975) provide an illustrative example of the different pharmacokinetics of a Type I compound (pentachloronitrobenzene) and a Type III halogenated aromatic compound (pentachlorobenzene) (see Table 2). They differ only in the presence or absence of a single polar nitro group. However, when dams were treated with 200 mg/kg of these compounds during days 6-15 of gestation, greatly different fat tissue residues resulted. On day 22 of gestation no pentachloronitrobenzene was found in maternal fat (detection limit 0.05 ppm) while fat levels of QCB were 3350 ppm. This is a difference in tissue residue levels of approximately 10^5. The polar molecule pentachloronitrobenzene is far more rapidly metabolized and excreted than the less polar molecule QCB.

4. The Three Tetrachlorobenzenes:

1,2,4,5-Tetrachlorobenzene has been the tetrachlorinated isomer most commonly used in industry. In 1975 production of 2,4,5-trichlorophenol (6 million pounds) and the herbicide 2,4,5-trichlorophenoxyacetic acid (10 million pounds) accounted for most of the production of 1,2,4,5-tetrachlorobenzene (Lewis, 1975). 1,2,4,5-Tetrachlorobenzene is also used as a fungicide, disinfectant, preservative for leather and textiles and as an intermediate in the production of hexachlorophene.

In separate studies rats were fed 0.5 to 500 ppm 1,2,4,5-tetrachlorobenzene for 28 days or 90 days (Chu et al., 1983, 1984a). Hepatic cytochrome P-450 based enzyme activities were increased by dietary levels of 50 ppm or higher.

In a teratology study, Kacew et al. (1984) found that 50 mg/kg of 1,2,4,5-tetrachlorobenzene increased both maternal blood cholesterol and several hepatic enzymes. Little or no teratogenic potential was seen in fetuses obtained from mothers treated with either of the three tetrachlorinated benzene isomers. In another study no adverse embryonic effects were found at a dose of 1,000 mg/kg/day of 1,2,4,5-tetrachlorobenzene in rats (Kitchin and Ebron, 1983c).

At 200 mg/kg/day 1,2,3,4-tetrachlorobenzene reduced the platelet count in pregnant rats and the number of fetuses per dam (Kacew et al., 1984). Administration of 300 mg/kg/day 1,2,3,4-tetrachlorobenzene reduced embryonic crown-rump and head length (Kitchin and Ebron, 1983b). The maternal livers showed enzyme induction without any decreases in maternal body weight. In a study with the third tetrachlorobenzene isomer, Kacew et al. (1984) found that 200 mg/kg/day of 1,2,3,5-tetrachlorobenzene reduced the number of fetuses per dam.

5. The Three Trichlorobenzenes:

1,2,4-Trichlorobenzene is the most commercially important of the three isomers. In 1973, 28 million pounds of this chemical were used principally as a dye carrier, herbicide intermediate and a heat transfer fluid (Lewis, 1975). 1,2,4-Trichlorobenzene is a stable non-corrosive compound with flame retardant and degreasing properties. Because of its high boiling point (210 °C), it is useful as a solvent for fractional crystallization of high melting point compounds. A commercial mixture of trichlorobenzenes useful for lubrication and as a dielectric fluid often contains 85% 1,2,4-trichlorobenzene, 7% 1,2,3-trichlorobenzene and trace amounts of other di- and tetrachlorobenzenes (Ware and West, 1977).

In Japanese quail or day-old chickens, 1,2,4-trichlorobenzene increased hepatic porphyrin content (Miranda et al., 1983). Chicken embryos or 18-day chickens were less sensitive to this biological effect. The minimal responsive dose was 50 mg/kg for quail and 200 mg/kg for day-old chickens. In quail, approximately 10-fold increases in delta-aminolevulinic acid synthetase activity and total hepatic porphyrin content were induced by treatment with 1,600 mg/kg of 1,2,4-trichlorobenzene (Miranda et al., 1983).

In rats, 1,2,4-trichlorobenzene caused hepatocellular hypertrophy. Rat liver enzymes of both the phase I (cytochrome P-450 and NADPH-cytochrome C reductase) and phase II classes (glutathione S-transferase and UDP-glucuronyl transferase) were increased by administration of 120 to 360 mg/kg/day of this chemical. Body weight decreases were seen with 360 mg/kg/day while death occurred in all experimental dams given 1,200 mg/kg day of 1,2,4-trichlorobenzene by gavage (Kitchin and Ebron, 1983a).

In rats given 360 mg/kg/day of 1,2,4-trichlorobenzene, day 14 embryos showed growth retardation in head length, crown-rump length, somite number and total embryonic protein. Specific abnormalities related to chemical treatment were not observed. This trichlorobenzene isomer did cause embryonic death which was statistically significant on a per embryo basis but not on a per litter basis (Kitchin and Ebron, 1983a).

Robinson et al. (1981) continuously exposed rats to 25-400 ppm of 1,2,4-trichlorobenzene in their drinking water for two generations. No adverse effects on fertility, growth, viability or locomotor activity were found. However, 1,2,4-trichlorobenzene is not expected to accumulate in maternal fat or milk as much as more fully chlorinated benzenes. Although 1,2,4-trichlorobenzene had no apparent estrogenic activity, it produced adrenal gland enlargement in the 95 day-old offspring at 400 ppm.

6. Hexachloro-1,3-butadiene:

Although hexachloro-1,3-butadiene (HCBD) is not currently produced in the United States, this compound is an unwanted by-product formed during the manufacture of trichloroethylene, tetrachloroethylene and processes associated with the chlorination of hydrocarbons (Laseter et al., 1975; Laska et al., 1976). HCBD has been used as a vineyard fumigant and was suggested to have potential use as a herbicide and insecticide (Laseter et al., 1975). In addition, HCBD has been used as a solvent for rubber, transformer liquid, hydraulic fluid and washing liquor for removing hydrocarbons (Verschueren, 1983). Thus, the potential sources for environmental pollution arise from industrial chemical processes and roadway runoff from rubber and hydraulic fluids. In the United States HCBD was identified as an organic contaminant in the water, soil and aquatic organisms such as crayfish of the lower Mississippi River (Laska et al., 1976). Similarly, HCBD was found in water and fish in the Rhine River and Great Lakes Basin (Goldbach et al., 1976; Konasewich et al., 1978). The knowledge that this contaminant is present in the environment has led investigators to examine whether HCBD may exert toxic effects on animals.

In adult rats HCBD is nephrotoxic. Morphologic evidence of HCBD-inflicted renal proximal tubule necrosis was reported by Ishmael et al. (1982). Ingestion of HCBD for a period of 2 years appeared primarily to produce kidney damage as evidenced by renal tubular hyperplasia and proliferation (Kociba et al., 1977c). In addition, functional alterations such as polyuria, glucosuria, enzymuria and decreased accumulation of p-aminohippurate were noted by several investigators (Berndt and Mehendale, 1979; Hook et al., 1982). HCBD did not markedly affect liver cell viability as serum glutamic pyruvic transaminase

activity was not significantly different from control values (Davis et al. 1980). Although the primary target tissue in acute studies is the kidney, chronic ingestion of HCBD in the diet was found to result in adrenal cortex adenocarcinomas, some of which metastasized to the lung (Kociba et al., 1977a, 1977c). Gage (1970) reported that inhalation of HCBD not only produced degeneration of renal proximal tubules but also caused enlargement and degeneration of adrenal tissue as well.

As acute exposure of adults to HCBD resulted in renal toxicity, subsequent studies of HCBD effects on reproductive capacity and neonatal survival were performed. To date no teratology studies of HCBD have been reported. Kociba et al. (1977b) maintained male and female rats on 2 or 20 mg/kg/day HCBD 90 days prior to mating, 15 days during and subsequently throughout gestation. HCBD appeared not to affect reproductive performance as evidenced by no effects on fertility, gestation, neonatal survival and morphology. In contrast, pathological examination of dams revealed renal tubular epithelial degeneration. Similarly, Harleman and Seinen (1979) maintained females for 4 weeks on either 150 or 1500 ppm HCBD and then placed these treated rats with untreated males for a further 3 weeks, during which time the contaminant was still present in the diet. In the 1500 ppm group no implantation sites were found. In contrast, there was no apparent effect on fertility or progeny in rats fed 150 ppm HCBD. While HCBD affected reproductive capacity in a concentration-dependent manner, renal histopathological changes were noted in the dams at both doses. Harleman and Seinen (1979) thus concluded that the main toxic action of HCBD is on the kidney. Additional support for the view that HCBD does not markedly affect reproductive performance was provided by Schwetz et al. (1974). In Japanese quail fed HCBD there was no apparent effect on egg production, fertility, hatchability of eggs, survival of hatched chicks,

eggshell thickness or histopathologic parameters. However, a positive control, HCB, was effective in reducing the survival of chicks hatched (Schwetz et al., 1974; Vos et al., 1971). In birds and animals, neither reproduction nor development are adversely effected by exposure to low amounts of HCBD.

Although HCBD seemed not to drastically affect the fetus, Poteryayeva (1966) reported the death of newborn rats from dams administered a single subcutaneous dose of 20 mg/kg of HCBD 3 months previously. Kociba et al. (1977c) found that 21 day-old rats were more susceptible than adults to the lethal effect of HCBD (Table 10). The LD50 in neonatal female and male rats was 65 mg/kg and 46 mg/kg, respectively, while in mature animals it was 580 mg/kg and 200-400 mg/kg, respectively. It is of interest that young (28 day-old) male rats were also more susceptible than adults to the nephrotoxic action of HCBD (Kuo and Hook, 1983; Hook et al., 1983). In a subsequent study Lock et al. (1984) demonstrated that young (21 day-old) mice were also more sensitive than adults to the nephrotoxic effects of HCBD. The finding that HCBD-induced renal damage was enhanced by phenobarbital in the young rats suggested that a metabolite and/or reactive intermediate was responsible for the observed nephrotoxicity (Hook et al., 1983). Thus susceptibility to nephrotoxicity by HCBD was greater in both young rats and mice than in adults (Hook et al., 1983; Lock et al., 1984). Why this susceptibility to HCBD-inflicted renal dysfunction decreases with age is at present unknown.

DISCUSSION

General Chemistry:

The toxicology of halogenated hydrocarbons can vary greatly from compound to compound. All halogenated hydrocarbons do not resemble DDT in their biological properties. The classification system developed by Matthews and

Kato (1979) is a useful first step in the comparison of biological effects of halogenated hydrocarbons. All the compounds covered in this article are Type III compounds, although some of their metabolites are Type I compounds. Some of the ten molecules discussed in this chapter differ considerably in their rate of oxidation and subsequent excretion. For example 1,2,4-trichlorobenzene is oxidized at a much higher rate than more fully chlorinated benzenes. This is a double edged sword, however. On the positive side 1,2,4-trichlorobenzene does not accumulate in Great Lakes fish to the extent chlorinated benzenes more resistant to oxidation do (e. g. 1,2,4,5-tetra-, penta- or hexachlorobenzene). 1,2,4-Trichlorobenzene is also excreted by mammalian systems much faster than higher chlorinated congeners (e. g. Table 4). On the negative side 1,2,4-trichlorobenzene is capable of substantial acute hepatic injury. Depletion of reduced glutathione content, cell death and a large increase in ornithine decarboxylase activity are all caused by 1,2,4-trichlorobenzene exposure. The formation of reactive intermediates depends on the number and position of the halogen substituents. The halogen-carbon bond is stronger than the hydrogen-carbon bond and more resistant to bond cleavage.

Pharmacokinetics:

In assessing the environmental effects and possible human exposure levels of this class of compound, the environmental levels, the n-octanol to water partition coefficient, and both the environmental and biological half-lives are all important points to consider. For example low water and sediment HCB concentrations become more concentrated higher up food chains as HCB possesses two undesirable characteristics 1) a high partition coefficient and 2) a long environmental and biological half-lives. Thus many humans have measurable HCB levels without having any known occupational exposure. Human tissue levels of HCB vary over

several orders of magnitude (Table 8). With the increased prevalence of breast feeding of infants, children will have an increased exposure to fat soluble toxins such as HCB.

Adult and Neonatal Toxicology:

In a structure activity study histological changes were found in liver, thyroid, kidney and lung following administration of tetra-, penta- and hexa-chlorobenzenes (Chu et al., 1983). In general terms all of the compounds produced similiar pathological changes. In the liver, cytoplasmic vacuolation, anisokaryosis and a reduction in the aggregated basophilia in the perivenous area were found. In the thyroid, these higher chlorinated benzenes caused an increase in epithelial height, angular collapse of thyroid follicles and a reduction in colloid density. Thyroid effects were the pathological finding which consistently occurred at the lowest doses, in fact, at every dose level tested. Eosinophilic inclusions were found in the renal proximal convoluted tubules of treated adult rats. In the lung, focal alveolar emphysema and alveolar mixed inflammatory cellular reaction were found. This study illustrates how similiar the toxicity of various compounds can be under certain exposure conditions.

In general, most chlorinated benzenes can cause porphyria. Rimington and Ziegler (1963) gave seven different chlorinated benzenes to male rats. Six compounds (mono-, o-di-, p-di-, 1,2,3-tri, 1,2,4-tri and 1,2,3,4-tetra-chlorobenzene) increased urinary porphyrins and their precursors while only 1,2,4,5-tetrachlorobenzene failed to do so under their experimental conditions.

In general, the acute central nervous system effects of the lower halogenated benzenes are similar to each other and to that of benzene. The high lipid solubility and relative chemical inertness of the parent molecule account for this similarity in short term nervous system effects. Either

central nervous system stimulation and/or depression may be seen. With lower chlorinated benzenes, more clinical information is available. For monochlorobenzene, the human nervous system symptoms of poisoning include headache, numbness, unconsciousness and muscle spasms. Acute exposures to o-dichlorobenzene have caused weakness, fatigue and headache in humans. p-Dichlorobenzene intoxication has caused dizziness, exhaustion, numbness, clumsiness and nausea in man.

Some of the low-dose health effects of orally administered chlorinated hydrocarbons in rats are presented in Table 11. No useful information on 1,2,3- or 1,3,5-trichlorobenzene was found. With dietary exposure to many of these compounds, an increase in liver enzymes or liver pathology was the biological effect observed at lowest doses. With HCB, slightly higher dietary levels resulted in neonatal pup death during lactation. With acute gavage exposure to other halogenated hydrocarbons, the number of fetuses per dam and fetal weight were found to be sensitive biological endpoints. As with chronic exposures, increases in liver enzymes and weight were sensitive markers to acute exposure to this family of compounds. HCBD caused renal pathology at low doses. The single acute oral LD50 values range from 756 (for 1,2,4-trichlorobenzene) to greater than 3,710 mg/kg (for OCS). This is a smaller difference than is seen with chronic exposure conditions. This is because the biological half-lives as well as the inherent toxicity of these molecules are so different.

In addition to the importance of metabolism and pharmacokinetics in determining the body burden and thus the neonatal toxicity of the halogenated hydrocarbons, a compound may have specific biological effects. HCBD causes renal toxicity and not hepatic changes as many halogenated hydrocarbons do. The reason for this is unknown. Similarly, the reason why QCB does not cause hepatic porphyria and virtually all the other chlorinated benzenes do remains a mystery of chemical specificity.

Summary: Some of the chemical and biological properties of ten of nonpolar halogenated hydrocarbons have been reviewed. Aspects ranging from chemistry and biochemistry on one hand to teratology and pathology have been presented in an attempt to form a multi-disciplinary view of their neonatal toxicology. There are more dissimiliarites and compound specificities among this family of compounds than are generally appreciated.

Abbreviations used:

HCB	hexachlorobenzene
HCBD	hexachlorobutadiene
OCS	octachlorostyrene
QCB	pentachlorobenzene
TCHQ	tetrachlorohydroquinone
SGPT	serum alanine aminotransferase

DISCLAIMER

The research desribed in this article has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

TABLE 1

Some Halogenated Organic Contaminants Identified in Great Lakes Region[a]

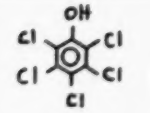
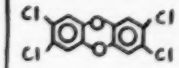
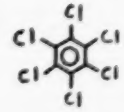
Chemical	Lake Site				
	Michigan	Superior	Huron	Erie	Ontario
Trichlorobenzenes	+	+	+	-	+
Tetrachlorobenzenes	-	+	+	+	+
Pentachlorobenzene	-	+	+	+	+
Hexachlorobenzene	+	+	+	+	+
Octachlorostyrene	-	+	+	+	-
Hexachlorobutadiene	-	+	+	+	-

[a]Organic substances present in fish and wildlife of Great Lakes Basin. This constitutes a partial list as the total number of all contaminants is far greater.

Adapted from (Konasewich et al., 1978)

TABLE 2

THREE CLASSES OF HALOGENATED AROMATICS

	Polarity	Polar Functional Group Present	Example	Organs with High Tissue Levels	Compound Requires Metabolism Before Excretion	Biological Procedures Important in Excretion	Approximate Biological Half-Life
Type I	High	Yes		Blood Liver Kidney	No	Renal Organic Acid Secretory System, Conjugation	< 1 day
Type II	Intermediate	Yes		Fat Liver	Yes	Oxidation, Conjugation	1-30 days
Type III	Low or Non-polar	No		Fat	Yes	Oxidation, Conjugation	> 30 days

Adapted from Matthews and Kato, 1979

TABLE 3

SOME CHLOROBENZENE CONCENTRATIONS IN THE GREAT LAKES AREA

	In Water (ppt)				In Sediments (ppb)		In Trout (ppb)	
	Niagara River	Drinking Water	Lake Ontario	Lake Huron	Lake Ontario	Lake Huron	Lake Ontario	Lake Huron
Trichlorobenzene								
1,2,3-	2.0	0.1	0.1	ND	7	0.3	0.2	0.2
1,2,4-	7.0	2.0	0.6	0.2	94	6.0	1.0	2.0
1,3,5-	2.0	ND	0.1	ND	60	0.7	0.3	4.0
Tetrachlorobenzene								
1,2,3,4-	3.0	0.3	0.1	0.05	33	1.0	2.0	4.0
1,2,3,5-	0.2	ND	ND	ND	6	0.4	0.2	0.5
1,2,4,5-	2.0	0.2	0.1	ND	52	1.0	1.0	2.0
Pentachlorobenzene	0.7	0.04	0.2	0.04	32	1.0	2.0	8.0
Hexachlorobenzene	0.2	0.1	0.06	0.04	97	2.0	25.0	61.0

Adapted from Oliver and Nicol, 1982

ND=Not detected

TABLE 4

RELATIVE URINARY EXCRETION RATES OF CHLORINATED BENZENES IN RABBITS

	Relative Urinary Excretion Rate
1,2,3-Trichlorobenzene	11
1,2,4-Trichlorobenzene	7
1,3,5-Trichlorobenzene	4
1,2,3,4-Tetrachlorobenzene	7
1,2,3,5-Tetrachlorobenzene	2
1,2,4,5-Tetrachlorobenzene	1
Pentachlorobenzene	2
Hexachlorobenzene	No urinary excretion found

Data is the sum of urinary glucuronides, sulphates, mercapturic acids and free chlorophenols and is expressed relative to the excretion rate of 1,2,4,5-tetrachlorobenzene. For this compound only 6% of the dose was excreted in the urine in 6 days.

Adapted from Williams, 1959

TABLE 5

BIOACCUMULATION OF SOME CHLORINATED AROMATIC HYDROCARBONS IN FEMALE RATS

	Feeding Level (ppm)	Days of Exposure	Female Rat Tissue Level (ppm) Liver	Fat	Ratio Fat Level/ Feeding Level	Ratio Fat Level/ Feeding Level Normalized to 1,2,3,4 TCB
Tetrachlorobenzene						
1,2,3,4 TCB	50	28	0.08	1.5	0.03	1
1,2,3,5 TCB	50	28	0.31	8.1	0.16	5
1,2,4,5 TCB	50	28	6.60	170.0	3.40	113
Pentachlorobenzene	50	28	1.3	12.0	0.24	8
	125	182	-	184.0	1.47	49
Hexachlorobenzene	50	28	70.0	426.0	8.52	284
	20	28	-	329.0	16.40	547
Octachlorostyrene	50	28	26.0	679.0	13.58	453

Adapted from:

Linder et al., 1980, 1983

Chu et al., 1983, 1982a

Table 6

Hepatic biochemical effects of equimolar doses of two chlorinated benzenes.

	Alkaline elution (fraction DNA eluted)	Ornithine decarboxylase (nmol CO2/ g liver/hr)	Glutathione (μmol/g)	Cytochrome P-450 (nmol/g)	Alanine amino-transferase (IU/ml)
Corn Oil	.200 ± .037 (8)	1.01 ± .26 (8)	5.93 ± .24 (8)	4.20 ± .24 (8)	13.82 ± .76 (8)
1,2,4,5-Tetra-chlorobenzene	.104 ± .030 (8)	3.28 ± 1.16 (8)	5.93 ± .29 (8)	4.47 ± .26 (8)	14.86 ± 1.32 (8)
1,2,4-Tri-chlorobenzene	.157 ± .032 (7)	135.7 ± 21.0*** (7)	3.12 ± .29*** (7)	5.18 ± .31* (7)	39.6 ± 7.8** (6)

Adult female rats were orally given 4.17 mmol/kg of the chlorinated benzene 21 and 4 hours before sacrifice. The fraction DNA eluted in the alkaline elution assay is a measure of DNA damage which includes alkylations and single strand breaks. The subcellular fractionation method used in this study gives a low yield of microsomal protein per g liver. Thus the cytochrome P-450 contents per gram liver reported here are lower than many other published values. Data is presented as the mean ± SEM. The number of adult female rats per group is in parentheses.

* P<.05
** P<.01
*** P<.001

TABLE 7 - SUMMARY OF EFFECTS OF OCS ON PREGNANT DAM AND FETUS

OCS (mg/kg/day)	Maternal			Fetus	
	Number at term	Number dead at day 21	Liver wt. (g)	Hydro-nephrosis	Small size
Control (0)	15	0	13.3±0.4	0	0
50	15	0	14.0±0.3	3	0
100	15	0	13.9±0.4	1	0
200	15	5	15.9±0.4[b]	2	0
400	15	9	17.3±1.2[b]	1	5

TABLE 8

HUMAN HCB LEVELS (ppb)

	Milk	Blood	Fat
A. Occupational Exposure			
Louisiana, USA (chlorinated solvent manufacture)	--	170.0	--
Spraymen, USA	--	40.0	--
Pest Control Operator, Australia	--	15.0	920
Australia	--	56.0	--
B. High Environmental Exposure			
Louisiana, USA	--	2.4	--
Australia	25.0	--	--
Urban Australia	28, 63	--	--
Rural Australia	79, 42	--	--
C. Environmental Exposure			
Upstate New York, USA	0.52	--	--
Louisiana, USA	--	0.1, 0.5	--
Canada	100.0	--	100
Germany	29.0, 0.15	--	6300
Germany	--	16.5	--
Australia	--	22.0	--
Papua, New Guinea	--	--	260
Norway	9.7	--	--
Japan	--	--	80, 210
Range of Reported Values	0.15-100.0	0.1-170.0	80-6300

Compiled from Courtney (1979), Currier et al., (1980), Curley et al. (1973), Siyali (1973), Williams et al. (1984), and Bush et al., (1985)

TABLE 9
PUP BRAIN HCB LEVELS AFTER CROSS FOSTERED HCB EXPOSURE

HCB Exposure Route		Twenty-One Day Rat
Placental	Lactational	Pup Brain HCB Level (ppm)
-	-	0.1
-	+	36.4
+	-	1.3
+	+	40.6

Dams were fed 80 ppm HCB from 14 days before mating to day 21 post-partum. Pups in the lactational exposure group received HCB via their mothers milk.

Adapted from Mendoza et al., 1977

TABLE 10 - SUMMARY OF STUDIES ON EMBRYOTOXICITY AND REPRODUCTIVE CAPACITY OF HCBD

Species	Dose	Reproductive Ability	Embryo-toxicity	Neonatal Toxicity	Reference
Japanese quail	0.3-300 ppm in diet for 90 days	-	-	N.D.	Schwetz et al, 1974
Rat	2 or 20 mg/kg for 2 years	-	-	N.D.	Kociba et al, 1977b
	150 ppm ir diet for 13 weeks	-	-	N.D.	Harleman and Seinen, 1979
	1500 ppm in diet for 13 weeks	+	+	N.D.	Harleman and Seinen, 1979
	single 20 mg/kg	N.D.	N.D.	+	Poteryayeva, 1966
	single 45 to 45 mg/kg	N.D.	N.D.	+	Kociba et al, 1977c

N.D.; not determined
-, no significant change
+, significant change

TABLE 11

LOW DOSE HEALTH EFFECTS OF SOME CHLORINATED HYDROCARBONS IN RATS

Compound	Rat Oral LD50	Gavage Dose mg/kg/day	Biological Effect	Dietary Exposure Level (ppm)	Biological Effect
1,2,4-trichlorobenzene[1]	756	10	↑ liver enzymes		
1,2,3,4-tetrachlorobenzene[2]	1470, 1167	200	↓ platelets ↓ number of fetuses	500	liver pathology
1,2,3,5-tetrachlorobenzene[2]	2297, 1727	200	↓ number of fetuses	500	liver pathology ↑ liver enzymes
1,2,4,5-tetrachlorobenzene[3]	3105, 1500	50	↑ blood cholesterol ↑ liver enzymes ↓ number of fetuses	50	liver pathology ↑ liver enzymes
pentachlorobenzene[4]	1125, 1080	200	↓ fetal weight	50	liver pathology
hexachlorobenzene[5]	3500	10	↑ liver enzymes	60 50	neonatal pup death liver pathology
octachlorostyrene[6]	>3710	200	↑ liver enzymes	50	↑ liver enzymes
hexachlorobutadiene[7]	504, 200	30	kidney pathology porphyria	150	kidney pathology

[1]Ware and West, 1977; Carlson and Tardiff, 1976
[2]Chu et al., 1983; Kacew et al., 1984
[3]Chu et al., 1984a; Ware and West, 1977; Kacew et al., 1984
[4]Linder et al., 1980; Chu et al., 1983; Khera and Villeneuve, 1975
[5]Ware and West, 1977; Carlson and Tardiff, 1976; Kitchin et al., 1982; Chu et al., 1983
[6]Kacew et al., 1986; Chu et al., 1984b, 1986
[7]Kociba et al., 1977b,c; Harleman and Seinen, 1979

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